Protein Kinase C Overactivity Impairs Prefrontal Cortical Regulation of Working Memory

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The prefrontal cortex is a higher brain region that regulates thought, behavior, and emotion using representational knowledge, operations often referred to as working memory. We tested the influence of protein kinase C (PKC) intracellular signaling on prefrontal cortical cognitive function and showed that high levels of PKC activity in prefrontal cortex, as seen for example during stress exposure, markedly impair behavioral and electrophysiological measures of working memory. These data suggest that excessive PKC activation can disrupt prefrontal cortical regulation of behavior and thought, possibly contributing to signs of prefrontal cortical dysfunction such as distractibility, impaired judgment, impulsivity, and thought disorder.

The prefrontal cortex allows us to appropriately guide our behaviors, thoughts, and emotions by using representational knowledge. Lesions of the prefrontal cortex produce symptoms of impulsivity, distractibility, and poor judgment. More extensive disruptions of prefrontal cortical function may also contribute to thought disorder (*1*) and hallucinations (*2*, *3*). Prefrontal cortical deficits are found in both bipolar disorder (*4*) and schizophrenia (*1*), illnesses worsened by exposure to stress (*5*, *6*) and recently associated with changes in PKC intracellular signaling (*7*–*10*).

PKC signaling is initiated by activation of phospholipase C releasing diacylglycerol (DAG), which subsequently binds to and activates PKC (Fig. 1A). Phorbol esters such as phorbol 12-myristate 13-acetate (PMA) activate PKC by acting as a long-lasting substitute for DAG (*11*); chelerythrine (CHEL) inhibits PKC activity by blocking this site. Once activated, PKC translocates from the cytosol to the plasma membrane and other subcellular compartments and undergoes autophosphorylation (p-PKC). Alpha-1 adrenergic receptors (α_1R) are coupled to PKC signaling by Gq proteins; thus, norepinephrine (NE, the endogenous ligand) and phenylephrine (PE, an α_1R agonist) indirectly activate PKC (Fig. 1A). We tested whether PMA or α_1R stimulation activates PKC in rat prefrontal cortical tissue (*10*). Because stress exposure increases NE release, activating α_1R and impairing prefrontal cortical cognitive function (*12*), we also examined the effects of the pharmacological stressor FG7142 on PKC activity. PMA, PE, and FG7142 significantly increased PKC activity (range: 23 to 40%, $P < 0.05$) in the membrane fraction of prefrontal cortical slices (Fig. 1B). Simultaneously, cytosolic PKC activity was decreased (range: 12 to 33%). Pretreatment with the PKC inhibitor CHEL completely blocked PMA, PE, or FG7142-induced increases in PKC activity in membranes (Fig. 1B). Acute, systemic administration of FG7142 to rats induced a significant increase (36.89% ± 13.5, $P < 0.05$) in PKCα levels (a PKC isoform associated with bipolar disorder; see SOM text) from frontal cortex membrane fractions (Fig. 1C) and a modest but not significant decrease in cytosolic PKCα (Fig. 1C), indicating comparable effects in vivo and in vitro.

The influence of PKC activation on cognitive function was tested in rats and monkeys performing spatial working memory tasks that depend on the prefrontal cortex (*10*). Successful performance of these tasks requires maintaining spatial information for a delay period, inhibiting inappropriate behavioral responses, and sustaining attention in the presence of distracters, all functions of prefrontal cortex. Rats were trained on the spatial delayed alternation task or on a control task, spatial discrimination, which has similar motor and motivational demands but depends on the posterior cortex rather than the prefrontal cortex. Rats received infusions of drug into the prefrontal cortex through surgically implanted cannulae. Local infusion of PMA significantly impaired performance of the delayed alternation task

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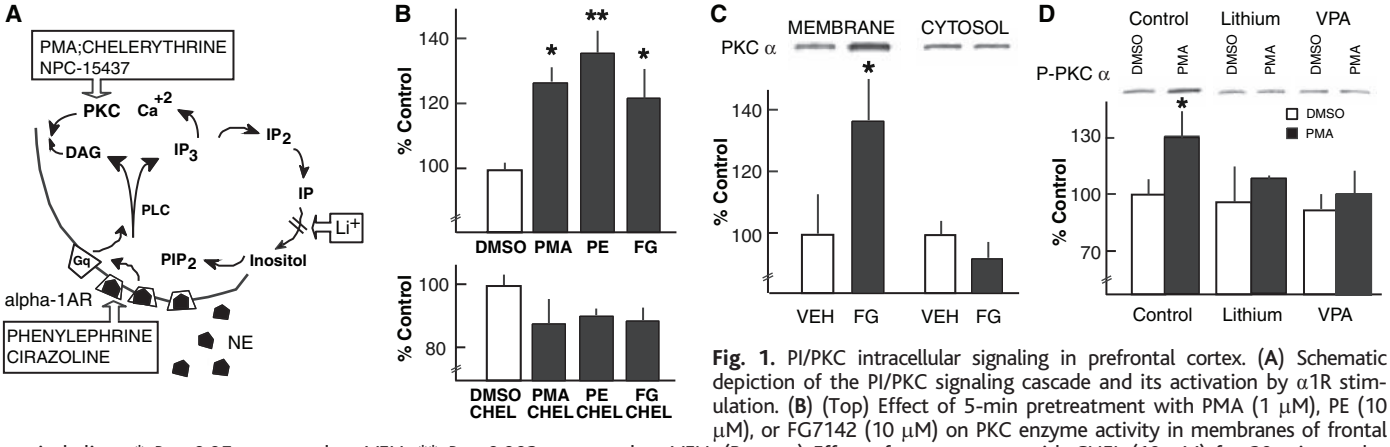

Fig. 1. PI/PKC intracellular signaling in prefrontal cortex. (**A**) Schematic depiction of the PI/PKC signaling cascade and its activation by α1R stimulation. (**B**) (Top) Effect of 5-min pretreatment with PMA (1 μM), PE (10 μM), or FG7142 (10 μM) on PKC enzyme activity in membranes of frontal cortical slices. * $P < 0.05$ compared to VEH; ** $P = 0.003$ compared to VEH. (Bottom) Effect of pretreatment with CHEL (10 μM) for 30 min on the increases in PKC activity induced by PMA, PE, and FG7142. All $P > 0.05$ compared to pretreatment with VEH+CHEL or VEH+VEH. (**C**) Effect of in vivo administration of the pharmacological stressor FG7142 (10 mg/kg) on the levels of PKCα in membrane and cytosolic fractions of rat frontal cortex. * $P < 0.05$ compared to VEH. (**D**) Chronic pretreatment with Li or VAL for 6 weeks abolished the PMA-induced increase in p-PKCα levels in rat frontal cortical slices. * $P < 0.05$ compared to VEH+VEH. IP, inositol phosphate; IP2, inositol biphosphate; IP3, inositol triphosphate; PIP2, phosphatidylinositol biphosphate.

(Fig. 2A and fig. S2). The PMA-induced working memory impairment was blocked by coadministration of CHEL at a dose that had no effect when administered alone (Fig. 2A). Control experiments indicated both anatomical and cognitive specificity: PMA infusion (–2.0 mm DV) into the cingulate and secondary motor cortex located dorsal to the prefrontal cortex had no effect on cognitive performance (fig. S3). However, PMA impaired delayed alternation performance when infused more ventrally (–4.5 mm DV) into the prefrontal cortex of the same animals (fig. S3). PMA (5.0 pg) infused into prefrontal cortex had no effect on performance of the spatial discrimination control task (fig. S4). Thus, the behavioral deficit was not due to nonspecific motor or motivational effects, which would alter both tasks. Instead, PKC activation selectively impaired the cognitive function of the prefrontal cortex.

Infusion of PE into the prefrontal cortex impairs working memory in both rats (*13*) and monkeys (*14*), and systemic injections of cirazoline (CIRAZ), an α_1R agonist that crosses the blood-brain barrier, impairs working memory in monkeys (*15*). Thus, we activated PKC indirectly by infusing PE into the prefrontal cortex in rats or by systemic administration of CIRAZ in monkeys. The PKC inhibitor CHEL was administered directly into the prefrontal cortex in rats or systemically in monkeys. Monkeys were trained on the spatial delayed-response task (*10*). As observed previously, α_1R agonist administration significantly impaired cognitive performance in both rats and monkeys (Fig. 2, B and C). This impairment was blocked by CHEL (Fig. 2, B and C), indicating that NE α_1R stimulation impairs working memory by activation of PKC. Together, these data demonstrate that either direct activation of PKC with a phorbol ester or indirect activation of PKC through α_1R stimulation impairs prefrontal cortical function.

Exposure to mild stressors, such as loud noise or low doses of the anxiogenic FG7142, impairs prefrontal cortical cognitive function in both humans and animals (*10*, *16*), and this impairment is prevented by α_1R antagonist pretreatment in animals (*12*). We tested whether stress-induced cognitive impairment is mediated by PKC. FG7142 impaired working memory in rats and monkeys, and this impairment was blocked by CHEL (Fig. 2, D and E). Infusions of CHEL into rat prefrontal cortex had no effect on stress-induced freezing or other noncognitive aspects of the stress response. Another PKC inhibitor, NPC-15437, also blocked the stress-induced cognitive impairment (fig. S5). Thus, endogenous (from stress) as well as exogenous (PMA) activation of PKC signaling has marked detrimental effects on prefrontal cortical function.

Lithium (Li) and valproate (VAL) are common treatments for patients with bipolar disorder. Although disparate in many of their actions, both agents attenuate PKC activity (*17*). We examined the effects of chronic treatment with Li or VAL (*10*) on PMA-induced p-PKCα in rat prefrontal cortical tissue. Li and VAL treatment for 6 weeks completely abolished the PMA-induced increase in p-PKCα (Fig. 1D). Li pretreatment prevents the working memory deficits induced by α_1R agonist infusion in rats (*13*). To test for this effect in monkeys, animals were pretreated with a dose of Li carbonate equivalent to that used to treat bipolar disorder (5.0 to 7.5 mequiv/kg, average blood levels of 0.61 ± 0.06 mequiv/L for the 7.5 mequiv/kg dose) followed by the α_1R agonist, CIRAZ. Li pretreatment prevented the CIRAZ-induced impairment in working memory performance (Fig. 2F). Similarly, pretreatment with 2.5 mg/kg VAL prevented the cognitive impairment induced by CIRAZ (fig. S6). Thus, like the selective PKC inhibitor CHEL, both Li and VAL protected prefrontal cortical cognitive function from α_1R-induced impairment.

Finally, we examined the influence of α_1R stimulation and PKC activation on prefrontal cortical function at the cellular level. Prefrontal cortical neurons fire during the delay period in a spatially selective manner as monkeys perform a spatial

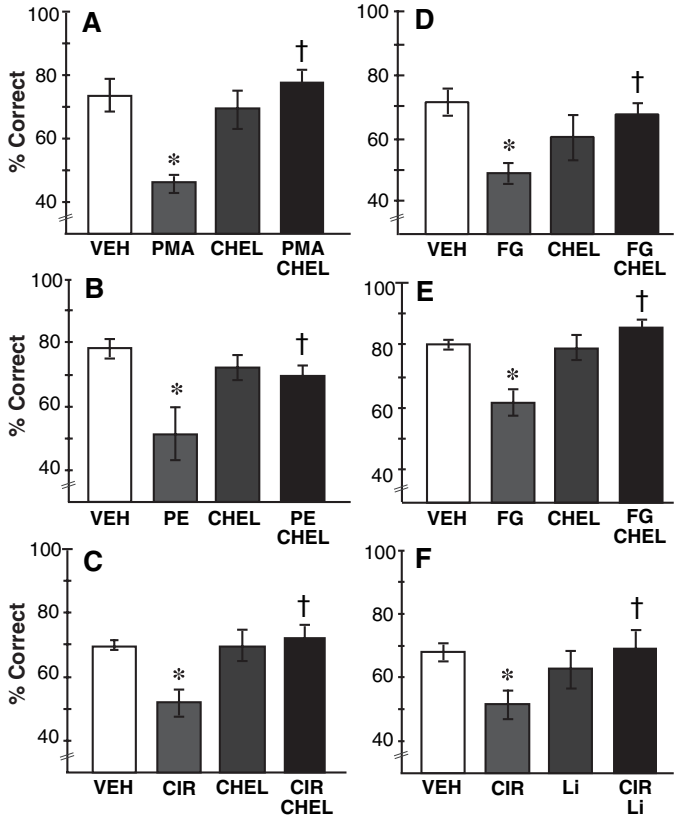

Fig. 2. Effect of PKC activation on prefrontal cortical cognitive performance in rats and monkeys. (**A**) Effect of direct modulation of PKC by infusion of PMA, CHEL (0.3 μg/0.5 μl), PMA+CHEL, or VEH directly into the prefrontal cortex on delayed alternation performance in rats (ANOVA-R; VEH+VEH versus PMA+VEH: *$F_{1,8}$ = 26.45, P = 0.001; PMA+VEH versus PMA+CHEL: †$F_{1,8}$ = 46.50, $P < 0.001$). VEH+VEH versus PMA+CHEL, $P > 0.05$. A dose of PMA was found for each individual animal that impaired delayed alternation performance (range: 0.05 to 25 pg/0.5 μl; representative dose/response curves in fig. S2). (**B**) Effect of indirect activation of PKC by infusion of PE (0.1 μg/0.5 μl), CHEL (0.3 μg/0.5 μl), PE+CHEL, or VEH directly into the prefrontal cortex on delayed alternation performance in rats (VEH+VEH versus PE+VEH: *$F_{1,8}$ = 11.10, P = 0.01; PE+VEH versus PE+CHEL: †$F_{1,8}$ = 8.01, P = 0.022). VEH+VEH versus PE+CHEL, $P > 0.05$. (**C**) The effect of indirect activation of PKC by systemic administration of CIRAZ (CIR), CHEL (0.03 mg/kg, orally), CIR+CHEL, or VEH on delayed response performance in monkeys. A dose of CIR (range: 0.001 to 10 μg/kg, intramuscular) was determined for each animal that reliably impaired delayed response testing (VEH+VEH versus CIR+VEH: *$F_{1,4}$ = 26.74, P = 0.007; CIR+VEH versus CIR+CHEL: †$F_{1,4}$ = 11.10, P = 0.008). VEH+VEH versus CIR+CHEL, $P > 0.05$. (**D**) The effect of the pharmacological stressor FG7142 (FG; range: 10 to 20 mg/kg, intraperitoneal), intraprefrontal cortex infusions of CHEL (0.3 μg/0.5 μl), FG+CHEL, or VEH on delayed alternation performance in rats (VEH+VEH versus FG+VEH: * ANOVA-R, $F_{1,10}$ = 25.095, P = 0.001; FG+VEH versus FG+CHEL: †$F_{1,10}$ = 10.170, P = 0.010). VEH+VEH versus FG+CHEL, $P > 0.05$. (**E**) The effect of very low doses of FG7142 (FG; range: 0.2 to 2.0 mg/kg), CHEL (0.03 to 0.15 mg/kg), FG+CHEL, or VEH on delayed response performance in monkeys (VEH+VEH versus FG+VEH: * $F_{1,5}$ = 20.69, P = 0.006; FG+VEH versus FG+CHEL: † $F_{1,4}$ = 21.23, P = 0.006). VEH+VEH versus FG+CHEL, $P > 0.05$. (**F**) Effect of Li pretreatment (5.0 to 7.5 mequiv/kg, orally, three times/day) on the response to cirazoline (CIR) in monkeys performing the delayed response task. A dose of CIR (range: 0.001 to 10 μg/kg) was determined for each animal that impaired delayed response testing (VEH+VEH versus CIR+VEH: * $F_{1,4}$ = 8.07, P = 0.047; CIR+VEH versus CIR+Li: † $F_{1,4}$ = 11.11, P = 0.029). VEH+VEH versus CIR+Li, $P > 0.05$.

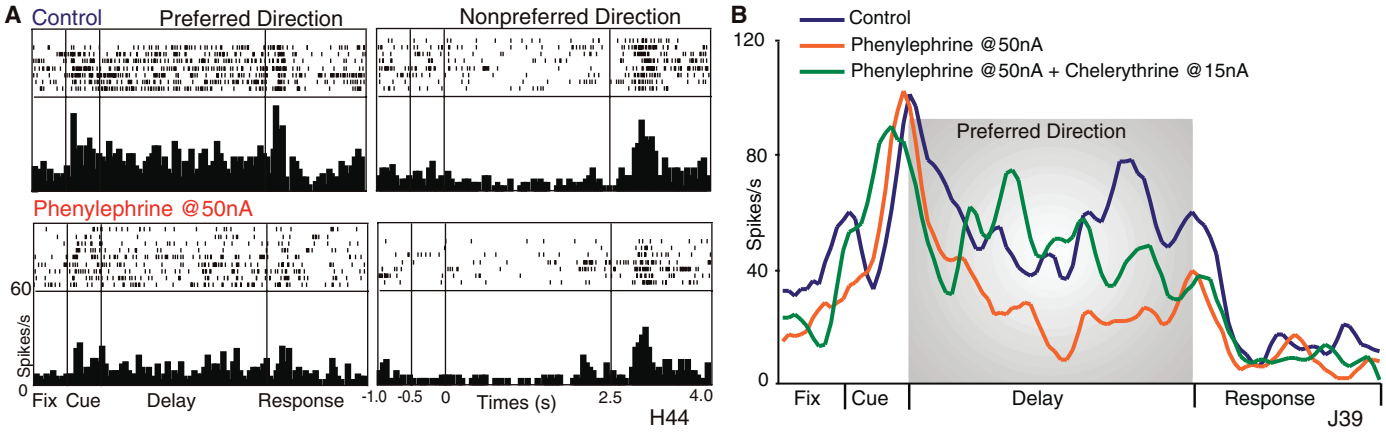

Fig. 3. Activation of PKC decreases the delay-related activity of prefrontal cortical neurons in monkeys performing a spatial working memory task. (**A**) Effect of iontophoretic application of the α_1R agonist PE on directionally selective delay-related activity in the oculomotor delayed-response task. Rasters and average histograms of Unit H44 during the control condition (top) and during PE iontophoresis (bottom) for preferred and nonpreferred directions are shown. (**B**) Effect of PE and CHEL on delay-related activity. Neuron J39 exhibited delay-related activity at its preferred direction during the control condition (blue). Iontophoresis of PE dramatically attenuated this activity (orange). Subsequent coapplication of CHEL with PE restored the delay activity (green).

working memory task (*6*). The effects of α_1R stimulation on memory-related firing were examined with single and multiple neuronal recordings in nonhuman primates performing a spatial-oculomotor delayed-response task (*10*). Twenty-eight neurons from the dorsolateral prefrontal cortex in two monkeys had sustained delay-related activity determined by two-way analysis of variance (ANOVA) with factors of task epoch versus baseline activity ($P < 0.01$). The activity from a representative neuron is shown in Fig. 3A. Iontophoretic application of PE (40 to 75 nA) attenuated delay-related activity in 25 out of 28 cases (one-way ANOVA for each neuron, $P < 0.01$), thereby reducing the cellular "memory" of the target location (Fig. 3A). As illustrated in Fig. 3A, PE (50 nA) significantly decreased delay-related activity for the neurons' preferred direction ($P < 0.0001$) but had no effect on activity recorded during trials for nonpreferred targets ($P > 0.05$). These data parallel previous findings that infusion of PE into monkey or rat prefrontal cortex impairs working memory performance (*13*, *14*). Co-iontophoresis of CHEL (15 nA) reversed the PE-induced reduction in delay-related activity in eight of nine neurons (one-way ANOVA for each neuron, $P < 0.001$; example shown in Fig. 3B; population response shown in fig. S7). Iontophoresis of CHEL by itself had no effect [three out of five cases (fig. S7)] or slightly reduced the delay-related activity (two out of five, one-way ANOVA, $P < 0.01$, data not shown). Thus, the reversal by CHEL was not due to independent additive effects of both agents. These findings indicate that PKC activation may impair mnemonic activity at the cellular level, thus providing a possible basis for the behavioral impairments observed in this study.

In summary, biochemical, behavioral, and electrophysiological data indicate that activation of PKC markedly impairs the cognitive functioning of the prefrontal cortex. These detrimental processes can be activated by exposure to uncontrollable stress, which is also known to exacerbate symptoms in patients with bipolar disorder (*5*) or schizophrenia (*6*). Dysregulation of the PI/PKC intracellular signaling cascade has been implicated in the etiology of bipolar disorder (*7*) and more recently in schizophrenia (*8*, *9*, and SOM text). Lead poisoning may also involve PKC overactivity (*18*) and has been associated with symptoms of inattention and hyperactivity (*19*). The current findings reveal a potential connection between dysregulation of PKC signaling and the symptoms of mental illness, demonstrating that overactivity of PKC can result in loss of prefrontal cortical regulation of behavioral response. Thus, high levels of PKC activity in the prefrontal cortex may contribute to a subset of symptoms involving the dysregulation of thought, affect, and behavior, which are features of many neuropsychiatric disorders.

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References

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